--56. (New) The method of claim 52, wherein the mitogen is a growth factor selected from the group consisting of epidermal growth factor, basic fibroblast growth factor, and combinations thereof.

--57. (New) The method of claim 52, wherein the mitogen is epidermal growth factor.

--58. (New) The method of claim 52, wherein the mitogen is basic fibroblast growth factor.

--59. (New) The method of claim 52, wherein the cells form a suspension culture.

REMARKS

Claims 26-27, 32-37, and 39-51 are pending in this case. Those claims are directed to methods for transplanting CNS neural stem cell progeny to a host. Applicants have amended claims 26, 32-34, and 36-40 to obviate the pending rejections and to clarify the claimed invention. The amendments add no new matter and are fully supported by the specification as filed.

Applicants have added new claims 52-59. New independent claim 52 recites a method of transplantation using an *in vitro* neural stem cell culture containing cells that can be proliferated in a culture medium supplemented with a mitogen, that retain the capacity for renewed proliferation, and that differentiate, under suitable culture conditions, to form neurons, astrocytes and oligodendrocytes. This claim, and dependent claims 53-59, are supported throughout the specification and add no new matter.

There are three remaining rejections in this case. The first rejection is under 35 U.S.C. § 112, second paragraph, for the recitation of the term "capable of differentiating," "biological agent," "substantially serum-free," and "said population of mammalian stem cells." The Examiner has provided suggestions for overcoming this rejection; the claims have been amended accordingly; and the rejection is moot.

The second rejection is under 35 U.S.C. § 112, first paragraph, for lack of enablement for the term "biological agent." Support in the specification for this term is demonstrated.

The third rejection is under 35 U.S.C. § 103(a), over various combinations of cited art. None of this art, taken alone or in combination, renders obvious the claimed invention, because that art does not and cannot refer to, teach, or suggest the CNS neural stem cell cultures recited in the methods of the instant claims.

The central nervous system neural stem cell cultures and the various methods and uses of those cultures claimed in this case and its related counterparts are pioneering inventions. The inventors have been widely recognized by the scientific community as the first to identify and isolate central nervous system neural stem cell cultures, and to teach methods for proliferating and differentiating those cell cultures, as well as various methods and systems using those cultures (such as the transplantation methods claimed here). Prior to Applicants' invention of central nervous system neural stem cell cultures, there was significant doubt as to the very existence of such cells. In fact, the operating dogma in neuroscience prior to Applicants' invention of central nervous system neural stem cells has been referred to as the "no new neurons" dogma. That dogma maintained that no new neurons were capable of being generated in the adult mammalian nervous system since there were no multipotent stem cells in the brain which could proliferate and subsequently differentiate into neurons. Applicants were the first to provide the art with precisely such neural stem cell cultures, and methods and systems for using them.

This case is one of several related co-pending applications that are based on the same specification. Of those cases, Serial No. 08/483,122 has issued as U.S. Patent 5,750,376 (claims directed to genetically modified central nervous system neural stem cell cultures), and Serial No. 08/486,648 (claims directed to central nervous system neural stem cell cultures) has been allowed. Both of these applications have the same filing date as the above-identified application. Both contain a specification identical to the above-identified application.

5851832

THE § 112, SECOND PARAGRAPH REJECTION

The Examiner has rejected under 35 U.S.C. § 112, second paragraph, alleging that the claims are indefinite in the recitation of the term "capable of differentiating", but has suggested certain language that would obviate the rejection. Applicants have amended the claims as

suggested by the Examiner. The claims now recite that progeny neural stem cells produce progeny that, under suitable culture conditions, differentiate - thus providing an assay for determining the differentiation state of the neural stem cells in culture. These amendments render the rejection moot.

The Examiner alleges that claim 36 is indefinite in the recitation of the term "substantially serum-free." Applicants have amended claim 36 to remove this term, rendering the rejection moot.

The Examiner alleges that claim 38 has insufficient antecedent basis for the term "said population of mammalian stem cells . . . in (a)." Claim 38 is not a pending claim.

The Examiner alleges that claim 40 has insufficient antecedent basis for the term "said differentiated cells." Applicants have amended claim 40 to depend from claim 39, rendering the rejection moot.

The Examiner also alleges that claim 27 is indefinite in the recitation of the term "biological agent." Applicants traverse this rejection. Claim 27 is directed not to "any and all" biological agents (as alleged in the Office Action, p. 4, para. 4), but to those biological agents selected from the Markush group of growth factors, growth factor receptors, neurotransmitters, neurotransmitter synthesizing genes, neuropeptides, and chromaffin granule amine transporter. Thus, the scope of the term "biological agent" is clear. Additionally, the term "biological agent" is further defined in the specification, p. 49, line 30, to p. 50, line 23. Thus, one skilled in the cell biological art would know, by referring to the specification, the scientific scope of the groups recited in claim 27. "If the scope of the subject matter embraced by the claims is clear, and if applicants have not otherwise indicated that they intend the invention to be of a scope different from that defined in the claims, then the claims comply with 35 U.S.C. § 112, second paragraph." MPEP § 2173.04.

Accordingly, Applicants respectfully request that the rejections under 35 U.S.C. § 112, second paragraph, be withdrawn.

THE § 112, FIRST PARAGRAPH REJECTION

The Examiner has rejected under 35 U.S.C. § 112, first paragraph, alleging that the claims are not enabled for a biological agent" because the specification does not enable genetically modifying neural stem cell progeny to express "any and all" biological agents (Office Action, p. 4, para. 4). Applicants traverse this rejection.

Claim 27 does not recite "any and all biological agents." Claim 27 is directed to biological agents selected from the group consisting of growth factors, growth factor receptors, neurotransmitters, neurotransmitter synthesizing genes, neuropeptides, and chromaffin granule amine transporter. The specification does enable one skilled in the art to practice the claimed method as recited.

The test of enablement is whether one skilled in the art could make or use the claimed invention from the disclosures in the patent coupled with information known in the art without undue experimentation. *Hybritech Inc. v. Monoclonal Antibodies, Inc.,* 802 F.2d 1367, 1384, 231 USPQ 81, 94, (Fed. Cir. 1986), *cert. denied,* 480 U.S. 947 (1987); MPEP § 2164.01. An extended period of experimentation may not be undue if the skilled artisan is given sufficient direction or guidance. Here, one skilled in the art is given sufficient direction and guidance to identify a biological agent in the specification, p. 49, line 30, to p. 50, line 23.

> The term 'biological agent' refers to any agent, such as a virus, protein, peptide, amino acid, lipid, carbohydrate, nucleic acid, nucleotide, drug, pro-drug or other substance that may have an effect on neural cells whether such effect is harmful, beneficial, or otherwise. . . . For example, the term may encompass certain neurotransmitters, neurotransmitter receptors, growth factors, growth factor receptors, and the like, as well as enzymes used in the synthesis of these agents. Examples of biological agents include growth factors such as FGF-1, FGF-2, EGF and EGF-like ligands, TGFα, IGF-l, NGF, PDGF, and TGFβ; trophic factors such as BDNF, CNTF, and glial-derived neurotrophic factor (GDNF); regulators of intracellular pathways associated with growth factor activity such as phorbol 12-myristate 13-acetate, staurosporine, CGP-4 1251, tyrphostin, and the like; hormones such as activin and TRH; various proteins and polypeptides such as interleukins, the Bcl-2 gene product, bone morphogenic protein (BMP-2), macrophage inflammatory proteins (MIP-lα, MIP-lβ and MIP-2); oligonucleotides such as antisense strands directed, for example, against transcripts for EGF receptors, FGF receptors, and the like; heparin-like molecules such as heparan sulfate; and a variety of other molecules that have an effect on

neural stem cells or stem cell progeny including amphiregulin, retinoic acid, and tumor necrosis factor alpha (TNFα).

The specification also provides examples of "biologically active substances."

Thus, the Applicants have claimed the method within the scope of the specifications and in light of the skill of a person knowledgeable in the arts. Accordingly, Applicants respectfully request that the rejections under 35 U.S.C. § 112, first paragraph, be withdrawn.

THE § 103 REJECTION

The Examiner has rejected the claims under 35 U.S.C. § 103(a) over Drago *et al.* (*Proc. Natl. Acad. Sci. USA 88(6)*: 2199-21203, 1991) (*"Drago"*) in view of Isacson *et al.* (*Exp. Brain Res. 75(1)*: 213-220, 1989) (*"Isacson"*) Applicants submit that the Office Action does not present a *prima facie* case of obviousness.

To establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. *In re Royka,* 490 F.2d 981, 180 USPQ 580 (CCPA 1974); MPEP ' 2143.03. Here, the cited references, either alone or in combination, do not teach neural transplantation using the "multipotent CNS neural stem cells" of the invention.

The development of the mammalian central nervous system (CNS) involves several stages of cellular development (*see* specification, p. 1, line 22, to p. 2, line 23). The first step is cell birth, in which stem cells and stem cell progeny (*i.e.*, daughter stem cells and progenitor cells which give rise to neuroblasts, glioblasts and new stem cells) proliferate. The second step is a period of cell type differentiation and migration when undifferentiated progenitor cells differentiate into neuroblasts and glioblasts which give rise to neurons and glial cells which migrate to their final positions. The third step in neural development occurs when cells acquire specific phenotypic qualities, such as the expression of particular neurotransmitters. The final step of CNS development is selective cell death, wherein the degeneration and death of specific cells, fibers and synaptic connections "fine-tune" the complex circuitry of the nervous system. The stages of differentiation are shown in Fig. 1 of the application, which is attached to this amendment.

In the first stage of development, the multipotent CNS neural stem cells proliferate. Stem cells have classically been defined as having the ability to self-renew (*i.e.,* form more stem cells), to proliferate, and to differentiate into multiple different phenotypic lineages. In the case of neural stem cells this includes neurons, astrocytes and oligodendrocytes. Thus, stem cells are "undifferentiated cells capable of a) proliferation, b) self-maintenance, c) the production of a large number of differentiated functional progeny, d) regenerating the tissue after injury, and e) a flexibility in the use of these options." Potten & Loeffler, *Development,* 110, p. 1001 (1990) (*see* specification, p. 3, lines 17-24).

Applicants were the first to provide the art with multipotent CNS neural stem cell cultures, the cells of which can be proliferated *in vitro* and whose progeny differentiate, under suitable culture conditions, into neurons (that express the recited neuronal markers) and glial cells (that express the recited astrocytic or oligodendrocytic markers). The specification, p. 11, lines 15-25, states the problem for transplantation procedures.

> The inability in the prior art of the transplant to fully integrate into the host tissue, and the lack of availability of cells in unlimited amounts from a reliable source for grafting are, perhaps, the greatest limitations of neurotransplantation. It would be more preferable to have a well-defined, reproducible source of neural tissue for transplantation that is available in unlimited amounts. Since adult neural tissue undergoes minimal division, it does not readily meet these criteria. While astrocytes retain the ability to divide and are probably amenable to infection with foreign genes, their ability to form synapses with neuronal cells is limited and consequently so is their extrinsic regulation of the expression and release of the foreign gene product.

The present application, not the prior art, teaches the use of multipotent CNS neural stem cell cultures to overcome this problem.

Drago

Drago does not disclose or teach multipotent CNS neural stem cell cultures. *Drago* refers to "neuroepithelial cells," obtained from E10 mouse embryos. but makes clear that those neuroepithelial cells have a limited ability to proliferate, and thus cannot self renew (*Cf.*, specification, p. 19, lines 27-30. The *Drago* neuroepithelial cells are made to survive and proliferate under the influence of mitogens ("Closer analysis shows that IGFs are acting primarily as survival agents and bFGF is acting as a proliferative agent." *Drago,* p. 2199, col 2, lines 6-7). The Office Action notes that "the method of Drago et al. does not enable the survival of their culture system beyond 3 days." Therefore, the *Drago* cells cannot be self-maintaining (a process that is necessarily longer than three days), in contrast to true "neural stem cells", such as the multipotent CNS neural stem cell cultures of the invention.

The Examiner alleges that neural stem cell cultures are an inherent property of the cells disclosed by *Drago.* Not true. Further, *Drago* does not disclose or teach that the preparation of E10 mouse neuroepithelial cells contained true CNS neural stem cells by any recognized criteria. *Drago* especially does not teach that the preparation of E10 mouse neuroepithelial cells can differentiate to neurons, astrocytes, and oligodendrocytes, in any event. Therefore, *Drago* is not and cannot be an enabling disclosure for the purpose of teaching transplantable CNS neural stem cell cultures.

Additionally, Applicants have amended the claims to recite that the neural stem cells differentiate to neurons, astrocytes, and oligodendrocytes under appropriate culture conditions. This property is not present in *Drago*.

Isacson

Isacson does not cure *Drago*'s failure to disclose multipotent CNS neural stem cell cultures. *Isacson* is cited to show transplanting of a fetal rat cell suspension from primordial striatum into lesioned caudate-putamen of the spinal cord of a primate model of Huntington's disease. *Isacson* prepared a suspension of primordial striatum from fetal rats (*Isacson,* p.214, col. 1, para. 3) in glucose saline solution. The Office action alleges that this cell preparation inherently contains neural stem cells. Applicants respond that obviousness cannot be predicated on what is unknown. *Isacson* made no attempt to culture these cells, and so did not demonstrate the presence of neural stem cells or neural stem cell cultures. Therefore Isacson is not an enabling disclosure for the purpose of teaching transplantable CNS neural stem cell cultures. Absent the understanding of Applicants' disclosure, the cell suspension of *Isacson* did not obviously contain neural stem cells.

Additionally, combining the *Drago* neural progenitor cells with the transplantation method of *Isacson* would not, with any reasonable expectation of success, result in transplanted CNS neural stem cell cultures. The problem for transplantation procedures is stated above and in specification, p. 11, lines 15-25. It is the Applicants, not *Drago* and *Isacson,* who provided the art with transplantable CNS neural stem cells, i.*e.,* cells that have been proliferated *in vitro* using mitogenic factors.

Lindvall

Claim 43 is rejected as being obvious over *Drago* and *Isacson* in view of Lindvall *et al.* (*Archives of Neurology 46(6):* 615-631, 1989) ("*Lindvall*"). *Lindvall* is cited to show neural transplantation into the recipient's striatum. However, *Lindvall* transplants dopaminergic neurons and neuroblasts obtained from the fetal human brain stems, cells which are differentiated cells. Dopaminergic neurons and neuroblasts are clearly not stem cells. Also, *Lindvall* does not culture the fetal neurons following dissociation and prior to implantation (*see Lindvall,* p. 617, col. 1). Further, *Lindvall* does not solve the problem faced by the art prior to Applicants' invention (*see,* specification, p. 10, line 18 to p. 11, line 18). *Lindvall* is the problem. Thus, *Lindvall* is not relevant to and does not enable transplanting CNS neural stem cells into a recipient's striatum.

E

Wendt

Claim 44 is rejected as being obvious over *Drago* and *Isacson* in view of Wendt *et al.* (*Exp. Neurology 79(2):* 452-461, 1983) ("*Wendt*"). The Office Action cites *Wendt* to show a cell transplant into a recipient's hippocampus. However, *Wendt,* like *Lindvall,* transplants differentiated neurons. Indeed, *Wendt* does not transplant neurons *per se,* but rather a "1- to 2-mm square piece of autologous sciatic nerve or fetal hippocampus" (*See Wendt,* p. 453, line 24-25). Note that sciatic nerve is not only not stem cell tissue, it is also not a CNS nerve. Thus, *Wendt* is not relevant to and does not enable transplanting CNS neural stem cells into a recipient's hippocampus.

Kesslak

Claim 45 is rejected as being obvious over *Drago* and *Isacson* in view of Kesslak *et al.* (*Exp Neurology 94(3):* 615-626, 1989) ("*Kesslak*"). The Office Action cites *Kresslak* to show neural transplantation to a recipient's frontal cortex. However, *Kresslak,* like *Wendt,* does not transplant neural cells *per se,* but rather tissue containing differentiated neurons. *Kresslak* surgically obtains rat frontal cortex tissue by dissection, with neither subsequent dissociation of the cells nor subsequent culturing of the frontal cortex tissue (*see Kresslak,* p. 617). Thus, *Kresslak* is not relevant to and does not enable transplanting CNS neural stem cells into a recipient's frontal cortex.

Andres

Claim 46 is rejected as being obvious over *Drago* and *Isacson* in view of Andres (*Neural Transplantation 1(1)*: 11-12, 1989) ("*Andres*"). The Office Action cites *Andres* for neural transplantation to a recipient's parietal cortex. *Andres* does not "transplant" anything; *Andres* surgically removes the parietal cortices of individual mice and then reimplants those parietal cortices back into the same mice (*see, Andres,* Title and Abstract). *Andres* is irrelevant to cellular differentiation. Thus, *Andres* does not make it possible to transplant CNS neural stem cells into a recipient's parietal cortex.

Price and Federoff

Claim 27 is rejected as being obvious over *Drago* and *Isacson* in view of Price *et al. (Development 104(3)*: 473-482, 1988) ("*Price*") and Federoff *et al. (Proc. Natl. Acad. Sci. USA 89(5)*: 1636-1640, 1992) (*Federoff*). *Federoff* is not available as a reference, because it has a publication date later than July 8, 1991, the application priority date.

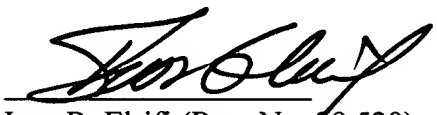
Price is cited to show genetically modified progeny. This citation is incorrect for two reasons. First, the *Price* cells are genetically modified *in vivo,* not *in vitro,* as recited in the claim. Second, *Price* does not identify CNS neural stem cells. *Price* identifies two classes of progenitor cells: (1) a class of cells that differentiates to form only gray matter astrocytes, and (2) a class of cells that differentiates to form neurons and a class of cells tentatively identified as glial cells of the white matter. By contrast, the multipotent CNS neural stem cell cultures of the invention differentiate to form neurons, astrocytes, and oligodendrocytes. Thus, *Price* does not cure *Drago and Isacson's* failure to teach CNS neural stem cell cultures.

In summary, the combination of the cited references does not render obvious Applicants' claimed methods. This is because none of these references, either alone or in combination, teach or suggest compositions comprising CNS neural stem cell cultures. *See,* Exhibits 1-11, enclosed herewith The Examiner has improperly ignored this evidence of patentability. Accordingly, applicants respectfully request that the rejections under 35 U.S.C. § 103 be withdrawn.

CONCLUSION

Applicants believe that this response addresses all remaining outstanding issues in this case. If the Examiner has any further issues, the Examiner is invited to contact the undersigned directly at (617) 348-1672. Accordingly, applicant requests that the Examiner enter the amendments and issue these claims.

Respectfully submitted,



Ivor R. Elrifi (Reg. No. 39,529)
John Prince (Reg. No. 43,019)
Attorneys for Applicants
c/o MINTZ LEVIN
One Financial Center
Boston, Massachusetts 02111
Telephone: (617) 542-6000
Telefax: (617) 542-2241

TRADOCS: 1130802.1 (_8j601!.doc)
10/28/98 10:46

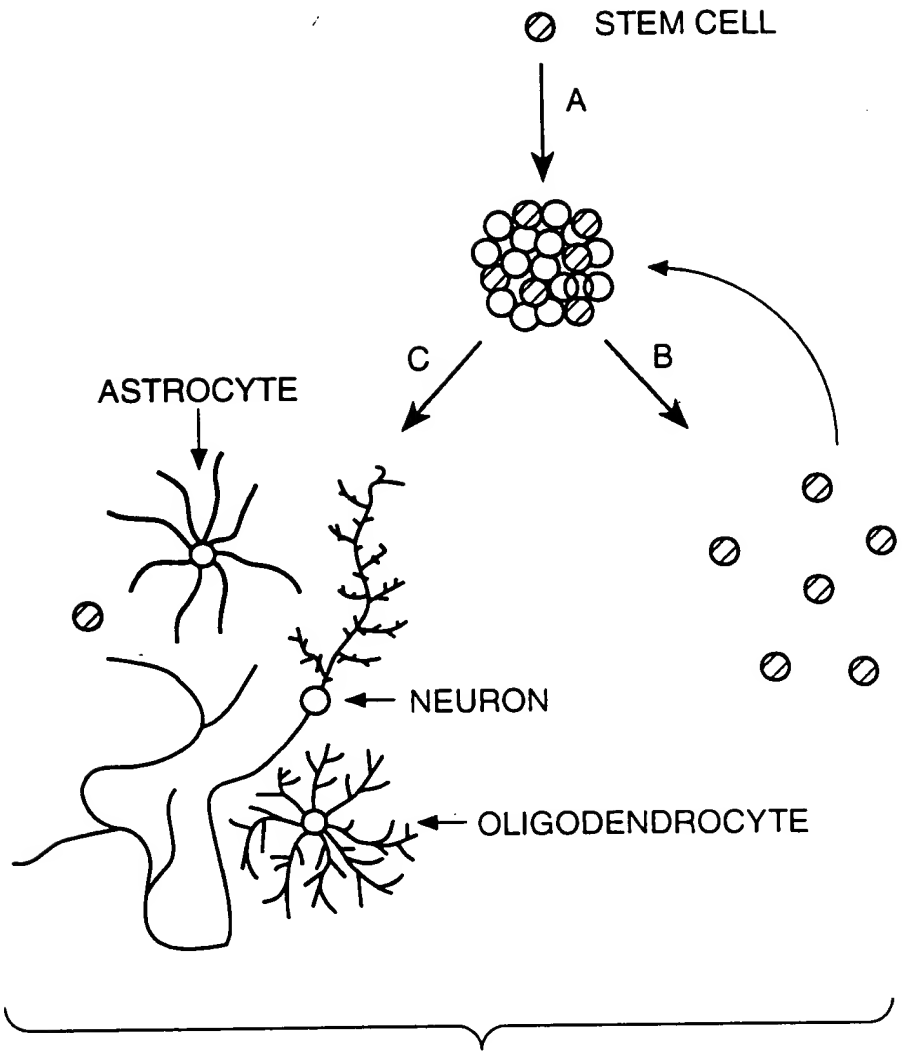


FIG._1